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# Membrane glycoprotein IV (CD36) is physically associated with the Fyn, Lyn, and Yes protein-tyrosine kinases in human platelets

*(FYN/YES/LYN/SRC)*

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ABSTRACT **Activation of platelets with thrombin and other agonists causes a rapid increase in the phosphorylation of multiple proteins on tyrosine. To identify candidate protein-tyrosine kinases (PTKs; EC 2.7.1.112) that may be responsible for these phosphorylation events, we analyzed the expression of seven Src-family PTKs and examined the association of these kinases with known platelet membrane glycoproteins. Five Src-related PTKs were detected in platelets: pp60$^{SRC}$, pp60$^{FYN}$, pp62$^{YES}$, pp61$^{HCK}$, and two *LYN* products of $M_r$ 54,000 and 58,000. The Fgr and Lck PTKs were not detected. Although strict comparative quantification of protein levels was not possible, pp60$^{SRC}$ was detected at higher levels than any of the other kinases. In addition, glycoprotein IV (GPIV, CD36), one of the major platelet membrane glycoproteins, was associated in a complex with the Fyn, Yes, and Lyn proteins in platelet lysates. Similar complexes were also found in two GPIV-expressing cell lines, C32 melanoma cells and HEL cells. Since PTKs appear to be involved in stimulus-response coupling at the plasma membrane, these results suggest that ligand interaction with GPIV may activate signaling pathways that are triggered by tyrosine phosphorylation.**

Platelets have been used as a model system to investigate eukaryotic signal transduction events. Platelet activation by agonists results in rapid changes in platelet morphology, cell aggregation, granule secretion, and involvement of the cell surface in coagulation reactions. Although platelet agonists have been shown to activate platelet signaling pathways, the mechanisms responsible for platelet activation are not definitely established. Recent evidence that thrombin and other agonists cause a rapid induction of the phosphorylation of multiple platelet proteins on tyrosine has raised the possibility that tyrosine phosphorylation may be involved in regulating changes in platelet physiology (1–7).

The protein-tyrosine kinase (PTKs; EC 2.7.1.112) responsible for agonist-induced tyrosine phosphorylation have not been identified. Since none of the known platelet receptors have been shown to possess intrinsic PTK activity, it is possible that members of the Src family of kinases may be activated by membrane receptors. Proteins in the Src family of PTKs lack a transmembrane domain, yet studies on one member of this family, pp56$^{lck}$, suggest that the Src family of kinases may form functional complexes with receptor proteins (for reviews see refs. 8 and 9). The Lck protein is associated in a noncovalent complex with the cytoplasmic domain of CD4 and CD8 proteins in T lymphocytes (10, 11). Antibody crosslinking of CD4 or CD8 causes a rapid activation of Lck kinase activity and an increase in tyrosine phosphorylation of several cellular proteins (12). It is predicted that other members of the Src family of PTKs associate with membrane receptors in other cell types.

Several members of the Src family of PTKs have been identified in human platelets. The most abundant PTK, pp60$^{SRC}$, represents approximately 0.2–0.4% of total cell protein (13). pp60$^{FYN}$ and pp62$^{YES}$ are also expressed at 1/5th to 1/10th of the Src protein (14, 15). In this study, we surveyed the PTK activity of seven (of eight known) members of the Src family of PTKs to identify candidate kinases that may be involved in platelet activation. Five kinases were detected: Src, Fyn, Yes, Hck, and two variants of Lyn. In addition, the membrane glycoprotein (GP)IV (CD36) was found to be physically associated with three Src-related PTKs: Fyn, Lyn, and Yes. Lastly, GPIV–PTK complexes were also found in two GPIV-expressing cell lines.

## MATERIALS AND METHODS

**Preparation of Washed Platelets.** Human platelets were isolated from venous blood by gel filtration as described previously (7) and adjusted to a concentration of $1 \times 10^9$ platelets per ml. The gel-filtered platelets were examined by flow cytometry to assess the degree of contamination of the platelets with leukocytes or erythrocytes. Flow cytometric dot plots of forward and right-angle light scatter demonstrated less than one erythrocyte or leukocyte per 10,000 platelets.

**Platelet Immunoprecipitation and Kinase Assays.** The platelets were lysed by incubation on ice for 30 min in an equal volume of 2× radioimmunoprecipitation assay (RIPA) buffer (1× = 1% Triton X-100/1% sodium deoxycholate/0.1% SDS/158 mM NaCl/10 mM Tris·HCl, pH 7.2) containing 5 mM NaEDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM $Na_3VO_4$ (Fisher), and Trasylol (FBA Pharmaceuticals, West Haven, CT) at 100 kallikrein inactivator units/ml and then clarified as described (13). Cell lysates were incubated at 4°C for 30–60 min with antibodies, then with activated Pansorbin (Calbiochem) or Pansorbin that had bound rabbit anti-mouse IgG (RAM) [for immunoprecipitations using monoclonal antibodies (mAbs)]. The immune complexes were washed three times in RIPA buffer with inhibitors, and either eluted with electrophoresis sample buffer (16) or incubated in 30 μl of kinase reaction mixture, which contains 5 μCi of [γ-$^{32}$P]ATP (1 Ci = 37 GBq), 1 μM ATP, 10 mM Tris·HCl at pH 7.2, 5 mM $MnCl_2$, and 5 mM $MgCl_2$, for 15 min at 4°C. The reactions were stopped by the addition of 500 μl of 1× RIPA buffer, and the phosphorylated proteins were eluted after centrifugation with Laemmli sample buffer, separated by electrophoresis on

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Abbreviations: GPI, GPIIa, etc., glycoprotein I, glycoprotein IIa, etc.; PTK, protein-tyrosine kinase; mAb, monoclonal antibody; RAM, rabbit anti-mouse IgG.

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an SDS/7.5% polyacrylamide gel as described (16), and detected by autoradiography.

**Cell Lines.** C32 cells were derived from a human melanoma (17) and HEL cells from human erythroleukemia cells (18). Both cell lines were kept as described (17, 18).

**Antibody Reagents.** Anti-Src mAb 327 was previously described (19). Polyclonal antisera directed against unique sequences from each Src-related protein were produced in rabbits immunized with multiple overlapping peptides corresponding to the following sequences from each protein: Fyn, amino acid residues 9–28; Yes, residues 5–71; Lyn, residues 18–62; Hck, residues 7–58; Lck, residues 39–58; and Fgr, residues 16–58. mAb 6F1 (specific for GPI/IIa) and mAb 6D1 (specific for GPIb) (20) were kind gifts from Barry Coller (State University of New York at Stony Brook). mAb 7E3 (specific for GPIIb/IIIa) (21) was a gift from Robert Jordon (Centocor, Malvern, PA). mAb $8A_6D_5$ and polyclonal antiserum anti-GP88 (specific for GPIV) were described previously (22). mAb IV.3, IgG2b [specific for Fc$\gamma$ receptor II (23)] was supplied by Lawrence Brass and Alan D. Schreiber (University of Pennsylvania, Philadelphia).

Platelet surface membrane glycoproteins were labeled with $^{125}$I by the lactoperoxidase/hydrogen peroxide method, as described previously (24). After labeling, platelets were lysed in RIPA buffer for immunoprecipitation studies.

## RESULTS

**Src-Related PTK Activity in Platelets.** To examine the expression of known members of the Src family of PTKs, platelet lysates were incubated with either polyclonal anti-peptide antibodies directed against unique sequences near the amino termini of the Fyn, Yes, Lck, Lyn, Fgr, or Hck proteins, or a monoclonal antibody (mAb 327) specific for Src. The proteins were detected by using an *in vitro* kinase assay that allows autophosphorylation of each PTK. Fig. 1 shows that these antibodies precipitated proteins corresponding to pp60$^{c\text{-}SRC}$, pp60$^{c\text{-}FYN}$, pp62$^{c\text{-}YES}$, and pp61$^{c\text{-}HCK}$, as well as two Lyn proteins, pp58$^{c\text{-}LYN}$ (Lyn$^U$) and pp54$^{c\text{-}LYN}$ (Lyn$^L$). The $M_r$ 54,000 and 58,000 proteins immunoprecipitated with the Lyn antiserum show electrophoretic mobilities similar to those of the two Lyn proteins detected by Yi *et al.* (25) and Stanley *et al.* (61) as products of alternatively spliced Lyn mRNAs. The Fgr and Lck proteins were not detected. Phosphoamino acid analysis revealed that each protein was phosphorylated on tyrosine (data not shown). In addition, proteins displaying electrophoretic mobilities and partial proteolytic peptide maps similar to those of the *in vitro*-labeled Src, Fyn, Yes, Lyn, and Hck were also immunoprecipitated from platelets labeled *in vivo* with [$^{32}$P]orthophosphate (data not shown). Although strict quantification of the relative proportions of each PTK was not feasible with the anti-peptide antibodies employed in these assays (because of differences in antibody affinity, $^{32}$P-labeling *in vivo*, and incorporation of $^{32}PO_4$ *in vitro*), labeled pp60$^{c\text{-}SRC}$ was detected at 5- to 10-fold higher levels than the other enzymes in each assay.

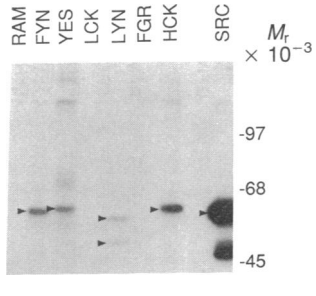


FIG. 1. Immune-complex protein kinase assay of Src-related gene products in human platelets. Src-related proteins were immunoprecipitated from platelet lysates by using antisera directed against the gene products indicated on the top of each lane, and incubated with [$\gamma$-$^{32}$P]ATP to allow autophosphorylation. For the RAM control lane, extracts were incubated with RAM alone. The proteins were separated on an SDS/7.5% polyacrylamide gel. The positions of the phosphorylated Src-related proteins are indicated by arrows; positions of molecular weight markers are shown at the right.

**Association of Platelet Membrane GPIV (CD36) with Src-Related PTKs.** We have previously shown that the induction of tyrosine phosphorylation of several platelet proteins is dependent on platelet aggregation mediated by fibrinogen binding to its receptor GPIIb/IIIa (7). To examine whether GPIIb/IIIa or other platelet membrane glycoproteins can physically associate with any PTK, several major platelet glycoproteins were selectively immunoprecipitated from platelet lysates and the immunoprecipitates were incubated with [$\gamma$-$^{32}$P]ATP to allow autophosphorylation of any associated PTKs (Fig. 2*B*). $^{125}$I-surface-labeled platelets were assayed in parallel as controls for the antibody immunoprecipitation (Fig. 2*A*). Antibodies to GPIa, -Ib, -IIb/IIIa, and -IV and the Fc$\gamma$ receptor II precipitated $^{125}$I-labeled proteins with the expected electrophoretic mobilities (see legend of Fig. 2 and ref. 30 for a review). In the samples incubated with [$\gamma$-$^{32}$P]ATP (Fig. 2*B*), only the GPIV and -Ia/IIa immunoprecipitates displayed proteins that were specifically phosphorylated *in vitro*. The three bands detected in the GPIV immunoprecipitates displayed electrophoretic mobilities sim-

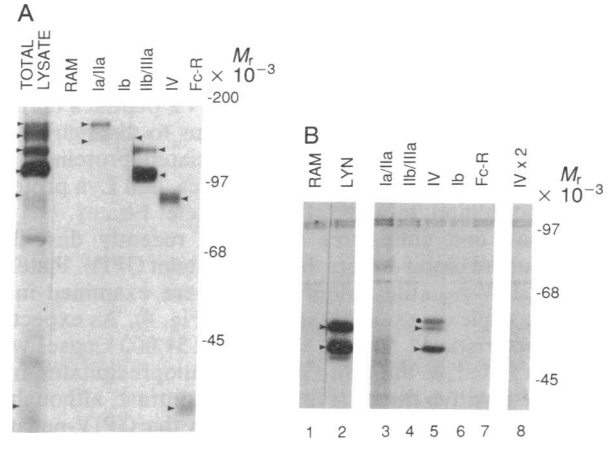


FIG. 2. Identification of kinases associated with platelet membrane glycoproteins. (*A*) Platelet membrane glycoproteins were labeled with $^{125}$I, solubilized in RIPA buffer, immunoprecipitated with antibody to the protein indicated on the top of each lane, and resolved in an SDS/7.5% polyacrylamide gel. The gel was dried and exposed to x-ray film for 2 days at −70°C. Total lysate represents the sample taken before immunoprecipitation. RAM indicates precipitation with control antibody. GPIa ($M_r$ 167,000) is associated in a noncovalent complex with GPIIa ($M_r$ 150,000) and functions as a collagen receptor (26–28). GPIb (with $M_r$ 140,000 and 24,000 subunits) associates with GPIX and functions as a receptor for von Willebrand factor (29). GPIIb/IIIa ($M_r$ 130,000 and 110,000, respectively) functions as a receptor for fibrinogen, fibronectin, vitronectin, and von Willebrand factor. A ligand for GPIV ($M_r$ 88,000–95,000, also known as GPIIIb, CD36, and PASIV) has not been definitely established (see *Discussion* for candidate ligands). The Fc$\gamma$ receptor II protein ($M_r$ 40,000) is a human IgG Fc binding protein (23). (*B*) Platelet lysates were prepared in RIPA buffer. Lanes 3–7, membrane glycoproteins were immunoprecipitated with antibodies to the proteins indicated above each lane, followed with *in vitro* kinase assay to allow autophosphorylation of associated kinases. Lane 1, RAM; lane 2, Lyn-specific antibody was used to precipitate this kinase as a mobility marker. Lane 8, IV × 2, lysate was cleared once with GPIV antibody, then incubated with fresh antibody to GPIV, and the washed immunoprecipitate was assayed *in vitro* for kinase. The $M_r$ 54,000/58,000 Lyn bands are indicated by arrowheads and $M_r$ 60,000 protein bands by a dot.

ilar to those of the two Lyn proteins (see lane 2) and to one, or several, of the $M_r$ 60,000–62,000 PTKs, Fyn, Src, Hck, and Yes. These three bands were detected by using either an mAb ($8A_6D_5$, lane 5), or a polyclonal antiserum (anti-GP88, data not shown) prepared against platelet GPIV (22). In addition, these PTK bands were not found in lysates that were cleared once with anti-GPIV ("IV × 2" reaction in lane 8). Multiple proteins were radiolabeled in the GPIa/IIa immunoprecipitates; however, since none of these proteins comigrated with the Src-like kinases, these proteins were not analyzed further.

**Characterization of the PTK–GPIV Complex.** To establish whether the 54,000–62,000 proteins radiolabeled in the GPIV immunoprecipitates were members of the Src family of PTKs, the reaction products from the *in vitro* immunocomplex kinase reaction were dissociated from the immunoadsorbent by boiling in 1% SDS (containing 10 mM Tris·HCl at pH 7.4 and 1 mM $Na_3VO_4$). The samples were then diluted 10-fold in a Triton X-100 containing buffer (buffer A), precleared to remove remaining immunoglobulin, and incubated with antibodies to each of the Src-like kinases expressed in platelets (Fig. 3). The Fyn, Yes, and Lyn proteins were immunoprecipitated from these reaction products. Although a faint $M_r$ 60,000 protein was detected in the Hck immunoprecipitates, the associated detection of the $M_r$ 58,000 and 54,000 Lyn-like proteins in this reaction raised concern that the Hck antiserum may have trapped proteins nonspecifically. The GPIV-precipitated proteins were also analyzed by partial proteolytic peptide mapping using V8 protease (data not shown). These maps confirmed that the $M_r$ 54,000 and 58,000 GPIV-associated bands represent the two Lyn proteins; however, the comigration of the V2 peptides derived from Fyn, Yes, and Src did not allow us to distinguish the identity of the $M_r$ 60,000 GPIV-associated proteins. The results in Fig. 3 indicate that the Fyn, Yes, and Lyn proteins coimmunoprecipitate with GPIV in platelet lysates.

Jamieson and colleagues (31) have recently described several human blood donors that lack platelet GPIV. Platelets from a GPIV-negative donor (V.B.) were examined in an immunocomplex protein kinase assay (Fig. 4). As expected, the characteristic $M_r$ 60,000, 58,000, and 54,000 kinases were phosphorylated in the anti-GPIV immunoprecipitates from the GPIV-positive donor (Fig. 4*A*). In contrast, although all of the platelet PTKs were expressed in the GPIV-negative donor, there was no detectable phosphorylation of those proteins in the anti-GPIV immunoprecipitates (Fig. 4*B*).

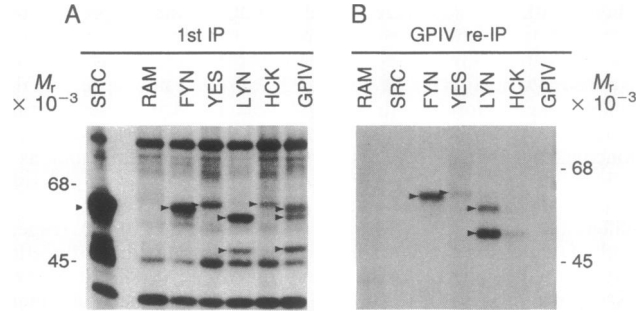


FIG. 3. Immunoprecipitate (IP) complex kinase assay to dissect GPIV complex in platelets. (*A*) The Src-related proteins or GPIV was immunoprecipitated from platelet lysates by using antisera against the protein indicated on the top of each lane (RAM = control) and incubated with [$\gamma$-$^{32}$P]ATP to allow autophosphorylation. (*B*) Duplicate GPIV immune complex kinase reactions (as in lane GPIV from *A*) were boiled with 1% SDS to dissociate the multiple components in the immune complex, diluted 10-fold in buffer A, and cleared with Pansorbin, and the supernatant was incubated with antibodies to each of the Src-family proteins as indicated on top of each lane. In both *A* and *B* the Src-related proteins are indicated with arrowheads.

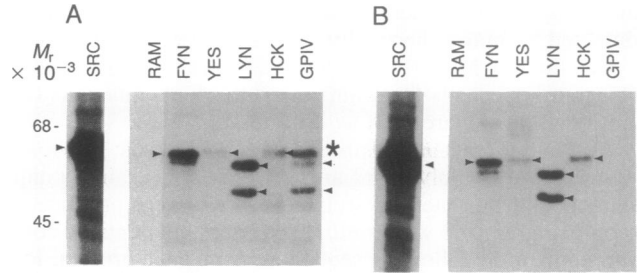


FIG. 4. Immune complex kinase assay of control and GPIV-negative platelets. Immune complexes were prepared and labeled *in vitro* with [$\gamma$-$^{32}$P]ATP. (*A*) Autoradiogram from a GPIV-positive control blood donor. (*B*) Autoradiogram obtained from a donor known to have GPIV-deficient platelets. The major Src-family PTK bands are indicated with arrowheads. The $M_r$ 60,000 PTK band coprecipitated with anti-GPIV is indicated with an asterisk.

These results indicate that the detection of Src-related PTKs in the anti-GPIV immunoprecipitates is dependent on the expression of GPIV, and precipitation of these kinases is not due to nonspecific binding to the GPIV antibodies. Platelets from a donor with Glanzmann thrombasthenia, which lack GPIIb/IIIa on the platelet surface, were also assayed for GPIV-associated kinases. The Fyn, Yes, and Lyn proteins were detected in the anti-GPIV immunoprecipitates at levels comparable to those seen in GPIIb/IIIa-positive donors (data not shown).

**PTK–GPIV Complex Formation in Other Cell Types.** To determine whether GPIV is associated with Src-like kinases in cells other than platelets, we analyzed the GPIV-associated kinase activity in two established cell lines that express GPIV (32–34): C32, a human melanoma cell line (17), and HEL, a human erythroleukemia cell line (18). HEL cells expressed high levels of the two Lyn proteins, low levels of Fyn and Yes, and barely detectable levels of Src (Fig. 5*B*). The major phosphorylated proteins precipitated with antibody to GPIV comigrated with the two Lyn proteins, with lower levels of a $M_r$ 60,000 phosphoprotein(s). The identity of the $M_r$ 86,000 phosphoprotein is not known. The protein migrates faster than $^{125}$I-labeled GPIV (data not shown) and could represent an additional member of the GPIV–kinase complex. C32 cells expressed high levels of Src and Fyn and lower levels of Yes. In GPIV immunoprecipitates, a single $M_r$ 60,000 protein was phosphorylated (Fig. 5*A*). Although the precise identity of the $M_r$ 60,000 proteins has not been established, that the profile of phosphorylated proteins was not altered by alkali treatment supports the possibility that this band represents at least one $M_r$ 60,000–62,000 Src-

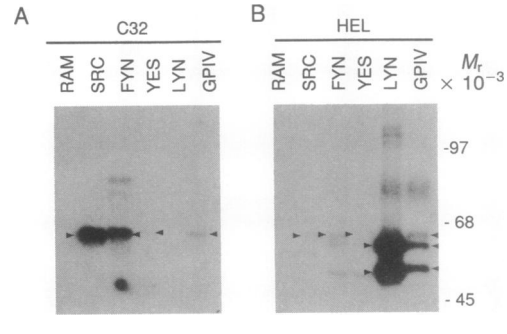


FIG. 5. GPIV-associated PTKs in C32 and HEL cell lines. Cells of C32, a melanotic melanoma cell line (*A*), or HEL, a human erythroleukemia cell line (*B*), were lysed with RIPA buffer and incubated with antibodies to the proteins indicated above each lane or with control RAM antibodies. *In vitro* kinase assays were carried out on the washed immunoprecipitates. The Src-related protein bands are denoted with arrowheads.

related PTK. These results indicate that the association of Src-related kinases with GPIV is not dependent on the platelet environment and that the complex does not require coordinate expression of Fyn, Yes, and Lyn, and they suggest that the ability of these kinases to bind GPIV is an intrinsic property of GPIV, or of another yet-unidentified protein that associates with GPIV in platelets, C32, and HEL cells.

## DISCUSSION

In this report, we identified five Src-related PTKs in platelets. The Src protein was detected at 5- to 10-fold higher levels than Fyn, Lyn, Yes, and Hck in assays involving *in vivo* labeling with $^{32}P$ and *in vitro* kinase assay of the immunoprecipitated PTKs. Three of these kinases—Fyn, Yes, and Lyn—were detected in immunoprecipitates containing the membrane glycoprotein GPIV, suggesting that these kinases are associated in a macromolecular complex with GPIV. In addition, Src-related PTKs were associated with GPIV in C32 melanoma cells and HEL erythroleukemia cells.

We and others have previously shown that activation of platelets with thrombin, collagen, ADP, ADP and epinephrine, platelet-activating factor, mastoparan, phorbol esters, and arginine vasopressin causes a rapid increase in the phosphorylation of several proteins (1–7). Since none of the known receptors for these agonists possess intrinsic PTK activity, the Src family of kinases represents candidate enzymes that may be activated by these diverse agonists. These kinases are located on the cytoplasmic face of cell membranes and could be activated as secondary events following ligand binding to transmembrane receptors. The interaction of the T-cell receptors CD4 and CD8 with the Lck tyrosine kinase serves as a model for such kinase/receptor interactions (reviewed in refs. 8 and 9). Three of the five PTKs detected in platelets, Src, Fyn, and Yes, are expressed in most tissues and hematopoietic cells; however, the levels of each protein vary in different cell types. The Hck protein has been previously identified primarily in hematopoietic cells of the myeloid lineage (35–37). The Hck protein expressed in mouse and human myeloid cell lines migrates as a single protein of $M_r$ 59,000, whereas that isolated from peripheral blood-derived human monocytes migrates as a triplet (8). Two alternative translational initiation sites for Hck have been identified: one protein initiates at the first ATG within exon 2, whereas the second, larger, form initiates at a CTG residue located 63 nucleotides upstream, generating a protein containing 21 additional amino acids (P. Lock and A. R. Dunn, personal communication). We observed that only a single form of Hck is expressed in platelets, possibly the larger form, since protein precipitated with Hck-specific antiserum migrated slightly slower than Src on SDS/polyacrylamide gels. Lyn is expressed in most types of hematopoietic cells, including macrophages, monocytes, B cells, natural killer (NK) cells, and basophils (8, 38). Analyses of Lyn cDNA clones (25, 61) have identified two alternatively spliced forms of Lyn mRNA, one lacking sequences encoding 21 amino acids within exon 2. The electrophoretic mobilities of the two forms of Lyn expressed in platelets are similar to those expressed in other hematopoietic cells (8, 25), consistent with the possibility that these two forms of Lyn are translated from alternatively spliced mRNAs.

The expression of several members of the Src family of PTKs in platelets raises the possibility that these enzymes may be involved in regulating multiple platelet signaling pathways. Activation of these enzymes may be mediated by direct interaction with membrane receptors (similar to that of CD4/Lck) or, perhaps indirectly, by downstream modifications of the enzymes by secondary or tertiary messengers. At least three of these PTKs (Fyn, Lyn, and Yes) appear to interact with one of the major platelet membrane glycoproteins, GPIV (Fig. 3). The specificity of this interaction was demonstrated by (*i*) the coprecipitation of GPIV and these kinases with both monoclonal and polyclonal antibodies to GPIV; (*ii*) the stable association between GPIV and these kinases in a stringent buffer (RIPA) containing a mixture of ionic and nonionic detergents; (*iii*) the absence of these PTKs in immunoprecipitates containing other abundant platelet glycoproteins (e.g., GPIIb/IIIa); (*iv*) the evidence that clearing of the platelet extract with antibodies to GPIV severely reduced, or abolished, the precipitation of associated kinases [although these kinases could be immunoprecipitated with antibodies to Fyn, Lyn, and Yes from the GPIV-cleared lysates (Fig. 2*B* and data not shown)]; and (*v*) the absence of these kinases in anti-GPIV immunoprecipitates from platelets lacking GPIV. In addition, PTKs with similar mobilities were found in association with GPIV in other cell types, including HEL and C32 cells.

The nature of the interactions between these proteins cannot be established from these studies. It is of interest that the COOH-terminal amino acids of GPIV contain a Cys-Xaa-Cys-Xaa$^+$-Xaa-Xaa$^+$-Xaa-Xaa-Lys sequence (34), which is similar to the sequence in CD4$\alpha$ (Cys-Xaa-Cys-Xaa-Xaa$^+$-Xaa$^+$-Xaa-Xaa-Lys) and CD8$\alpha$ (Cys-(+)-Cys-Xaa-Xaa$^+$-Xaa-Xaa-Xaa-Lys) (in which Xaa$^+$ represents a basic amino acid and Xaa, any amino acid) and is essential (and sufficient in the case of CD8$\alpha$) for the interaction between Lck and the two T-cell receptors CD4$\alpha$ and CD8$\alpha$ (39, 40). Since two cysteine residues within the $NH_2$-terminal region of Lck are essential for interaction with CD4/CD8, it has been proposed that the association between these proteins involves a tetrahedral metal complex (reviewed in refs. 8 and 9). Since Fyn, Yes, and Lyn do not share a similar Cys-Xaa-Cys sequence, it is possible that another, yet-unidentified, protein is part of this complex.

The ability of GPIV to associate with three kinases in the Src family suggests that GPIV, or some other associated protein, binds to a site that is shared between Fyn, Lyn, and Yes. This interaction could involve sequences within the SH2 or SH3 domains in the $NH_2$-terminal half of the PTKs, or possibly conserved residues in the SH1 catalytic domain (41). The absence of Src from this complex is perplexing, since this kinase is so abundant in platelets and is homologous to Fyn, Lyn, and Yes sequences in the SH1, SH2, and SH3 domains. It is possible that Src's accessibility to the GPIV complex is blocked by its association with other platelet proteins, or that Src has a lower affinity for GPIV (CD36). Of note in this regard, we have detected a small amount of Src (less than Yes) in GPIV immunoprecipitates from platelet extracts prepared in a buffer containing 1% Triton X-100 (without deoxycholate and SDS; data not shown). However, the significance of this weak interaction is unclear.

GPIV is expressed in several other cell types *in vivo*, including monocytes [GPIV referred to as CD36 (42–44) or OKM5 antigen (45)], endothelial cells (46, 47), and mammary secretory epithelial cells [GPIV referred to as PASIV (48)]. GPIV (CD36) is the receptor for cytoadherence to erythrocytes infected with the malaria parasite *Plasmodium falciparum* (22, 32, 34, 49–52). Adherence to endothelial cells is believed to play an important role in the pathogenicity of malaria, by preventing parasite clearing in the spleen and contributing to the occlusion of small vessels in the lethal syndrome of cerebral malaria (49–51). The role of GPIV in platelets has not been definitely established. Several lines of evidence indicate that GPIV is a site for divalent cation-dependent binding of the adhesive protein thrombospondin (43, 47, 53, 54), and it has been proposed that thrombospondin, which is the major $\alpha$-granule protein secreted from platelets, serves as an extracellular bridge between GPIV and the fibrinogen-liganded form of GPIIb/IIIa complex [through thrombospondin affinity for fibrinogen (55)]. This interaction

is hypothesized to stabilize platelet aggregates, leading to the formation of irreversible macroaggregates (56, 57). Such a clustering of GPIV with GPIIb/IIIa, or perhaps other membrane glycoproteins, could be necessary for activation of the GPIV-associated kinases. We have previously shown that thrombin induction of tyrosine phosphorylation of two platelet proteins ($M_r$ 84,000 and 95,000–97,000) is dependent on binding of fibrinogen to GPIIb/IIIa, as well as the subsequent platelet aggregation (7). This result is consistent with the possibility that activation of tyrosine phosphorylation requires membrane protein clustering, perhaps involving GPIIb/IIIa and the GPIV–PTK complex, which could take place during the process of platelet aggregation.

Tandon and coworkers (33, 58) have also reported that GPIV is a receptor for $Mg^{2+}$-independent binding of collagen fibrils to platelets. The expression of recombinant clones of GPIV in cells that lack thrombospondin and collagen receptors should establish whether GPIV alone, or in combination with other membrane proteins, serves as a receptor for these adhesive proteins.

The association of GPIV (CD36) with PTKs implies that this receptor can potentially trigger intracellular signaling pathways. Binding of anti-GPIV antibodies or cytoadherent *P. falciparum*-infected erythrocytes has been shown to induce a respiratory burst in monocytes and Fc-dependent aggregation and secretion in platelets (44, 52, 59, 60). These results suggest that GPIV (CD36) engagement may activate intracellular pathways that induce changes in cell physiology. These pathways would appear to be dispensable for normal platelet functions, since GPIV-negative donors have no detectable bleeding defects (31). Since platelets employ multiple pathways to mediate essential functions, it is possible that GPIV-negative platelets may use alternative pathways that compensate for the loss of GPIV. There may be an activation of Fyn, Yes, and Lyn following agonist treatment of platelets. Further investigations of the role of tyrosine phosphorylation in these processes may reveal new insights into the physiological function of protooncogenes in normal cells, as well as the mechanisms involved in events regulated by cell–cell and cell–matrix interactions.

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